In Vivo Interactions between Opioid and Adrenergic Receptors for Peripheral Sympathetic Functional Regulation

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Methionine enkephalin (ME) produced a variety of pharmacological and/or physiological actions.[1] It decreased blood pressure (BP) although its cardiovascular actions are variable depending on routes of its administration, state of anesthesia, and animal species tested.[2] Its hypotensive action was blocked by naloxone, but not by naloxone methobromide, a quaternary analog. Hypotension was also antagonized by adrenergic α-receptor antagonists such as phentolamine.[3] ME suppressed sympathetic nerve activity before the reduction in pressure.[4] ME also affected blood flow and vascular resistance in selected organ beds of rabbits.[5] Systemic infusion of ME increased norepinephrine in microdialysis dialysate in ventrolateral medulla in rats.[6] The primary objective of this work was to elucidate the nature of opioid and adrenergic receptor interactions, which will eventually modify peripheral cardiac function.

MATERIALS AND METHODS

Sprague-Dawley (SD) or spontaneously hypertensive rats (SHR) were anesthetized with intraperitoneal Inactin (120 mg/kg). The right femoral artery and vein were cannulated for monitoring BP and drug infusion. Body temperature of the animals was maintained and their heads were fixed on stereotaxic frame. Cardiovascular parameters were recorded as described previously.[4] For intracisternal and ventrolateral medulla (C_1 area) microinjection the atlanto-occipital membrane was punctured by a 26-gauge needle that was mounted to a stereotaxic micromanipulator as reported.[6] ME (0, 1, 3, and 10 μg/kg) was injected into the cisterna magna in a volume of 5 μL. Obex (stereotaxic zero) was visualized, and C_1 coordinates were 2.0 mm anterior, ± 1.9 mm lateral, and 3.0 mm below the floor of the fourth ventricle as reported.[6] Catecholamines were determined by high-performance liquid chromatography as previously reported.[6] For the assay of thyrosine hydroxylase (TH), dopamine β-hydroxylase (DβH) and phenylethyl *N*-methyl transferase (PNMT), adrenal glands and C_1 area (punch biopsy) were homogenized in 1 mL and 0.25 mL of 5 mM

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potassium phosphate buffer (pH 7.0), each containing 0.2% Triton X-100. Homogenates were centrifuged at 10,000 × *g* for 10 min. The resultant supernatants were used for enzymes assay, based on protein concentration. For the DβH assay [^{14}C]SAM (sulfur adenosyl methionine) and 7.5 × 10^{-5} M Ca^{2+} were used with an appropriate volume of the supernatant. For the PNMT assay 25 μL of C_1-area supernatant, and 2 μL of adrenal supernatant were used at 37°C for 15 min in the presence of [^{3}H]SAM.

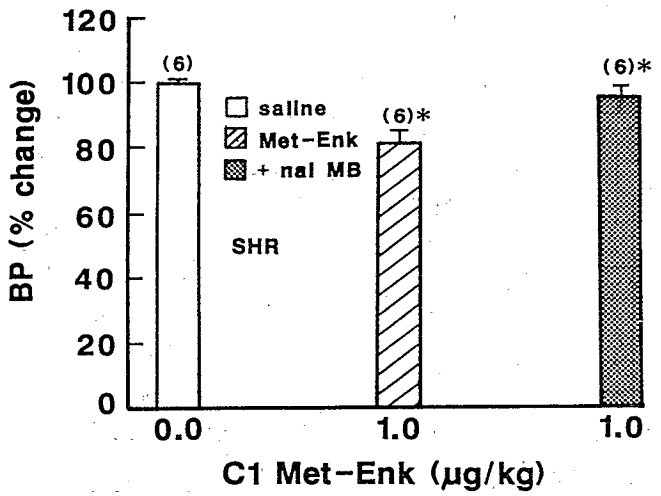

FIGURE 1. Effects of methionine enkephalin (Met-Enk) alone and after the administration of naloxone methobromide (nal MB) in spontaneously hypertensive rats (SHR). Young adult SHR rats were anesthetized with intraperitoneal Inactin (120 mg/kg). The ventrolateral medulla (C_1 area) was exposed as reported[6] on stereotaxic instrument. Met-Enk was injected into the area at a dose of 0 (control) or 1 μg/kg in a volume of 1 μL. In a different animal the procedure was repeated 10 min after an intravenous administration of nal MB (1.3 mg/kg). Systolic blood pressure was recorded as reported previously.[5] Control pressure was 116 ± 8 mmHg, which was considered 100%. The number in parentheses indicates the number of rats used and the vertical bars represent SEM. Asterisk indicates significance ($p \leq 0.05$).

RESULTS AND DISCUSSION

Intravenous (i.v.) and intra C_1 administration of ME reduced blood pressure, which was blocked by naloxone pretreatment. Intracisternal injection of ME had little effect at tested dose (30 μg/kg). Pretreatment of the animal with an intravenous naloxone methobromide did not protect the animal. As little as 1 μg/kg ME administration at C_1 produced hypotension in SD rats and in SHR (FIG. 1). Pretreatment of the animal with naloxone increased adrenal TH activity (6.4 versus 9.4 nm/mg protein per 20 min at 30 °C) and adrenal PNMT activity significantly (data not shown), suggesting that the enzyme activities might be suppressed in normal conditions. However, the PNMT and TH activities at C_1 area were not affected after

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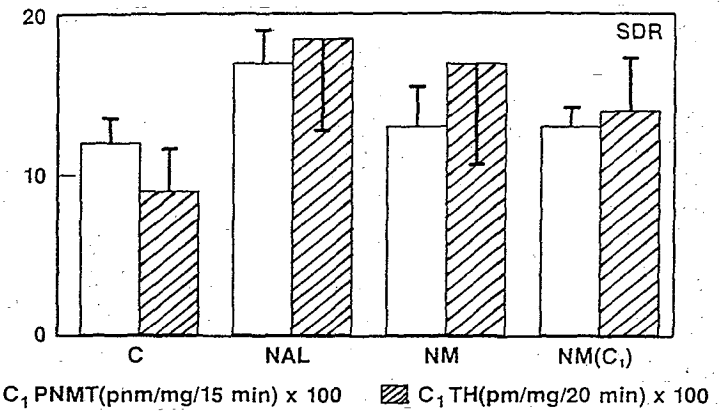

FIGURE 2. Phenylethyl *N*-methyltransferase (PNMT) and thyrosine hydroxylase (TH) activities at the ventrolateral medulla (C_1 area) in Sprague-Dawley rats. C_1 areas were obtained by punch biopsy, and the enzyme activity of PNMT and TH was assayed in the homogenates of the biopsy samples as described in MATERIALS AND METHODS. The PNMT and TH activities in the samples obtained from the animals that were treated with 1 mg/kg naloxone (NAL) were slightly higher than the saline treatment as control (C); however, the differences were not significant. Naloxone methobromide (NM) treatment intravenously or directly into the C_1 area [NM(C_1)] did not change the enzyme activities. Each point represents mean of at least four to seven tissues collected from three to five animals.

naloxone (1 mg/kg, i.v.), naloxone methobromide (1.3 mg/kg, i.v.), which was applied directly into C_1 area in both SHR and SD rats (FIG. 2). Furthermore, DβH activity at C_1 area was not affected by treatment of the animal. It is concluded that specific interactions between opioid and adrenergic receptors for catecholamine synthesis, and its metabolism and release in both central and peripheral systems may dictate cardiac function such as blood pressure regulation.

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